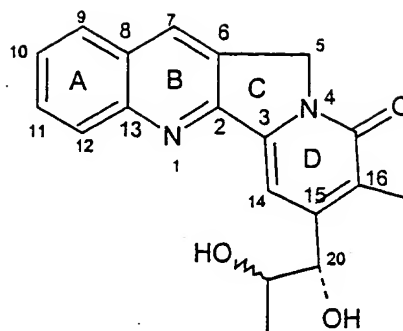
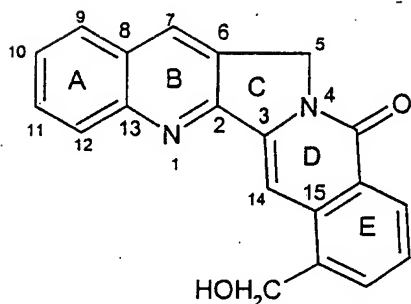
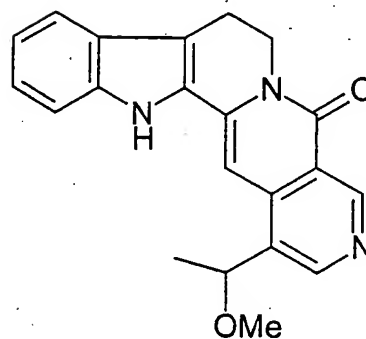


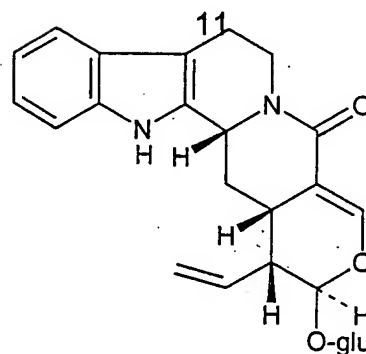
- 1  $R_1=R_2=H, R_3=OH$
- 2  $R_1=R_3=OH, R_2=H$
- 3  $R_1=OCH_3, R_2=H, R_3=OH$
- 4  $R_1=H, R_2=R_3=OH$
- 5  $R_1=H, R_2=OCH_3, R_3=OH$
- 6  $R_1=R_2=R_3=H$
- 7  $R_1=R_2=H, R_3=O(CH_2)_4CH_3$
- 8  $R_1=OCH_3, R_2=H, R_3=O(CH_2)_4CH_3$



10



9



12

Diagrams of the chemical structures of major natural camptothecin and its analogs in *Camptotheca acuminata*: camptothecin (1), 10-hydroxycamptothecin (2), 10-methoxycamptothecin (3), 11-hydroxycamptothecin (4), 11-methoxycamptothecin (5), 20-deoxycamptothecin (6), 20-hexanoylcamptothecin (7), 20-hexanoyl-10-methoxycamptothecin (8), 22-hydroxyacuminatine (9), 19-hydroxymappicine (10), 19-O-methylangustoline (11), and vincoside-lactam (12).

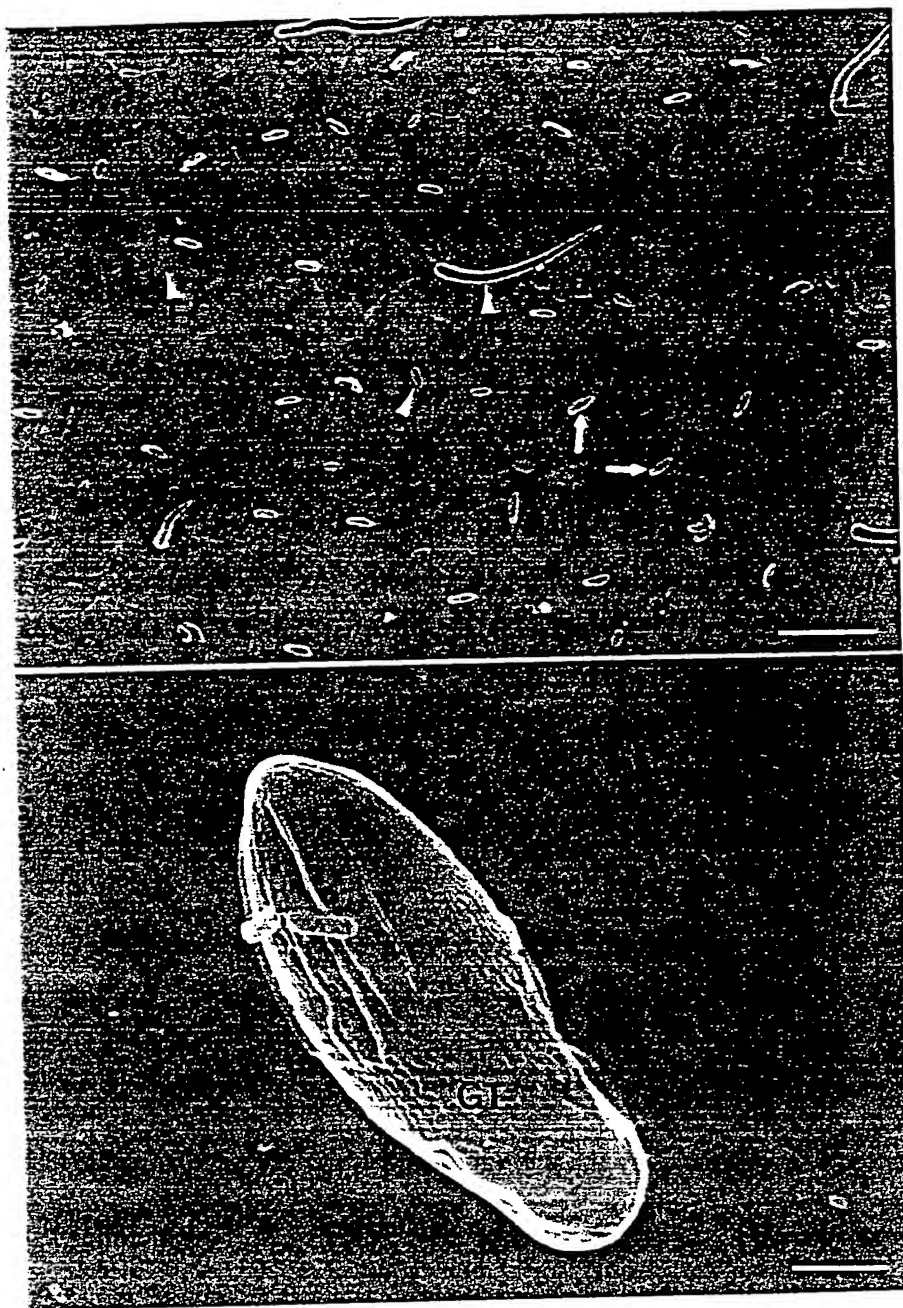
**Fig. 1.**

		Young Tissue	Intermediate Tissue	Old Tissue
Leaf	Definition	<1 week old	1-4 week old	>4 week old
	CPT % (fw)	0.05140 ± 0.00869	0.022450 ± 0.00135	0.01018 ± 0.00169
Stem	Definition	<4 week old	= 2 year old	= 5 year old
	CPT % (fw)	0.01063 ± 0.00313	0.00795 ± 0.00036	0.00648 ± 0.00078
Stem Wood	Definition	<1 year old	= 2 year old	= 5 year old
	CPT % (fw)	0.00662 ± 0.00014	0.00309 ± 0.00007	0.00566 ± 0.00027
Stem Pith	Definition	<1 year old	-----	-----
	CPT % (fw)	0.01433 ± 0.00053	-----	-----
Stem Bark	Definition	<1 year old	= 2 year old	= 5 years old
	CPT % (fw)	0.01380 ± 0.00257	0.01960 ± 0.00054	0.01010 ± 0.00313
Flower/Fruit	Definition	<1 week old (flower)	= 8 week old (fruit)	= 16 week old (fruit)
	CPT % (fw)	0.02276 ± 0.00280	0.01127 ± 0.00039	0.05058 ± 0.00294
Root	Definition	<4 weeks	-----	> 4 weeks
	CPT % (fw)	0.00171 ± 0.00005	-----	0.00526 ± 0.00105

Note: Shiyu Li, et al. unpublished.

**CPT distribution in different tissues of Camptotheca acuminata** (Seed source: SFA 94-03; leaf, stem, and root materials were collected in May, wood and bark samples were collected in August and fruit samples were collected in June, August, and October, respectively) (mean ± s.d.) (on the basis of fresh weight).

**Fig. 2**



a. [Top] Scanning electron micrograph of surface view of lower leaf epidermis of *Camptotheca Lowreyana* 'Katie'. Scale Bar = 100  $\mu$ m.

b. [Bottom] Scanning electron micrograph of mature glandular trichome (GT) on lower leaf surface of *Camptotheca Lowreyana* 'Katie'. Scale Bar - 5 $\mu$ m.

**Fig. 3**

Species/Variety	Average Glandular trichome length (μm)	Average Glandular trichome width (μm)	Average Glandular trichome Density (μm)	Young Leaves CPT Concentration (% ± s.d.)	Old Leaves CPT Concentration (% ± s.d.)
<u>C. acuminata</u>	34.87 ± 3.92	13.64 ± 1.97	46.80 ± 5.26	0.05822 ± 0.01654	0.01607 ± 0.00204
<u>C. yunnanensis</u>	35.20 ± 2.70	13.72 ± 1.27	27.10 ± 11.40	0.05443 ± 0.01499	0.1308 ± 0.00217
<u>C. lowreyana</u>	46.49 ± 4.51	10.94 ± 1.42	81.50 ± 6.50	0.08423 ± 0.01541	0.02021 ± 0.0321
<u>C. lowreyana</u> <u>'Katie'</u>	42.46 ± 3.34	14.64 ± 1.68	65.50 ± 10.41	0.10641 ± 0.01612	0.02174 ± 0.00167
<u>C. lowreyana</u> <u>'Hicksii'</u>	56.32 ± 6.72	14.02 ± 1.87	69.20 ± 14.45	0.12284 ± 0.01089	0.02632 ± 0.00278

Note: Shiyou Li, et al. unpublished.

**Glandular trichome size and density on lower leaf surfaces and CPT concentration in leaves of Camptotheca (on the basis of fresh weight.)**

**Fig. 4**

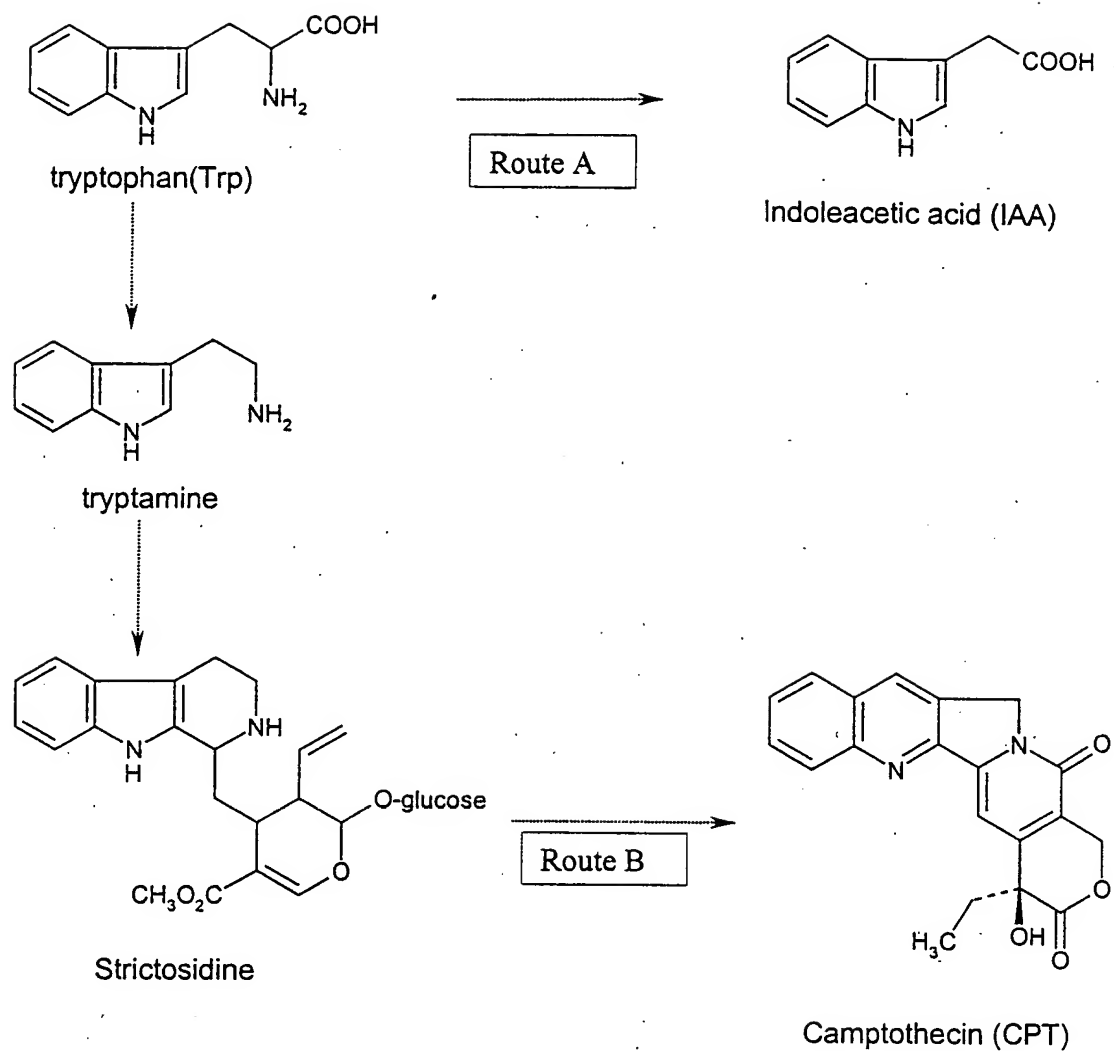
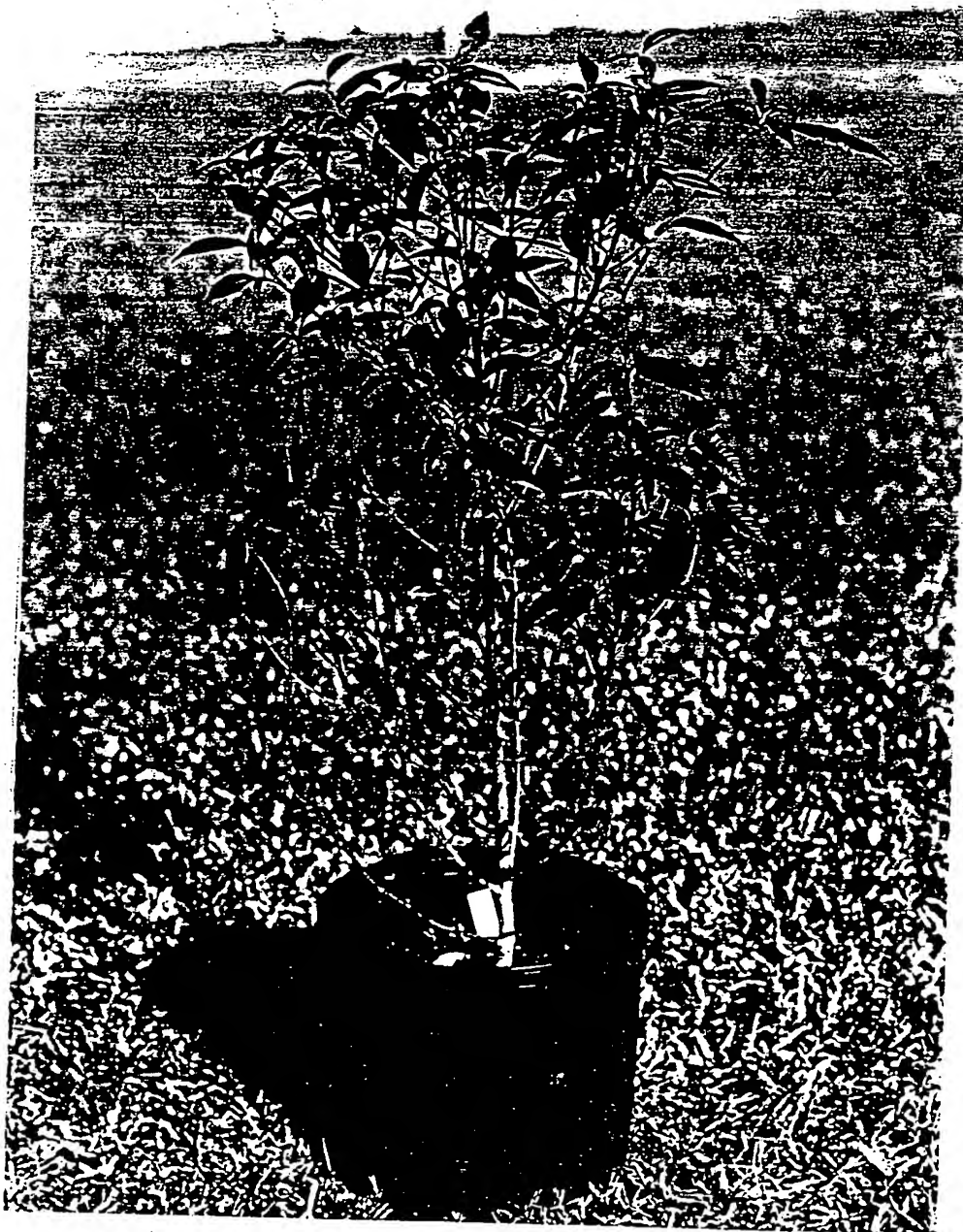


Diagram of two biosynthetic pathways showing tryptophan (TRP) as a biosynthetic precursor for both indoleacetic acid (Route A for stimulating growth) and camptothecin (Route B for inhibiting growth).

**Fig. 5**



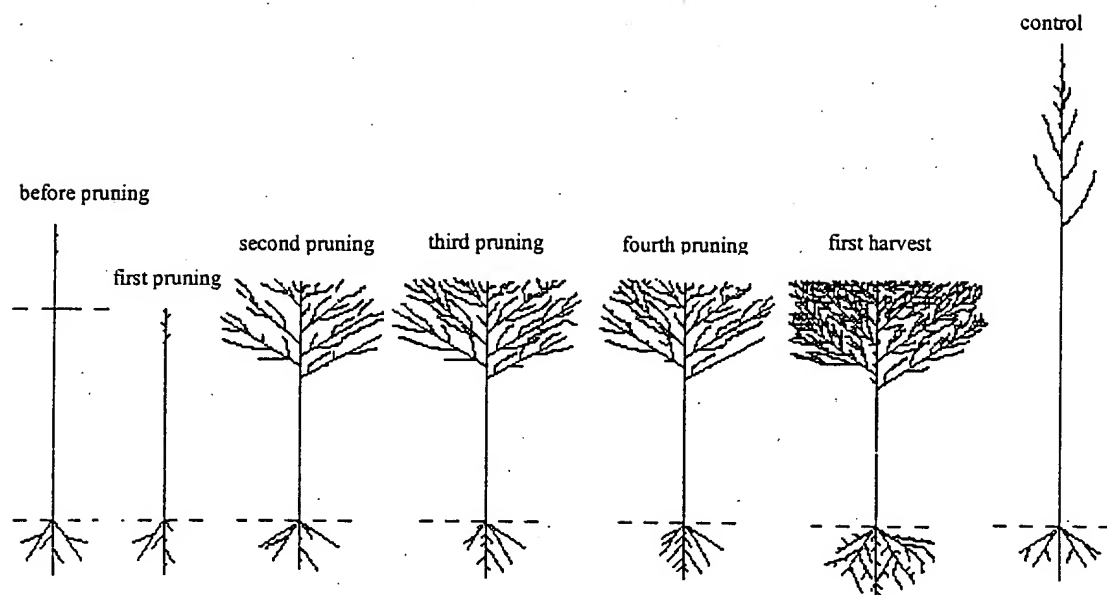
Photograph of Camptotheca lowreyana 'Katie'.

**Fig. 6**



Drawing of a Camptotheca leaf after leaf-tip pinching.

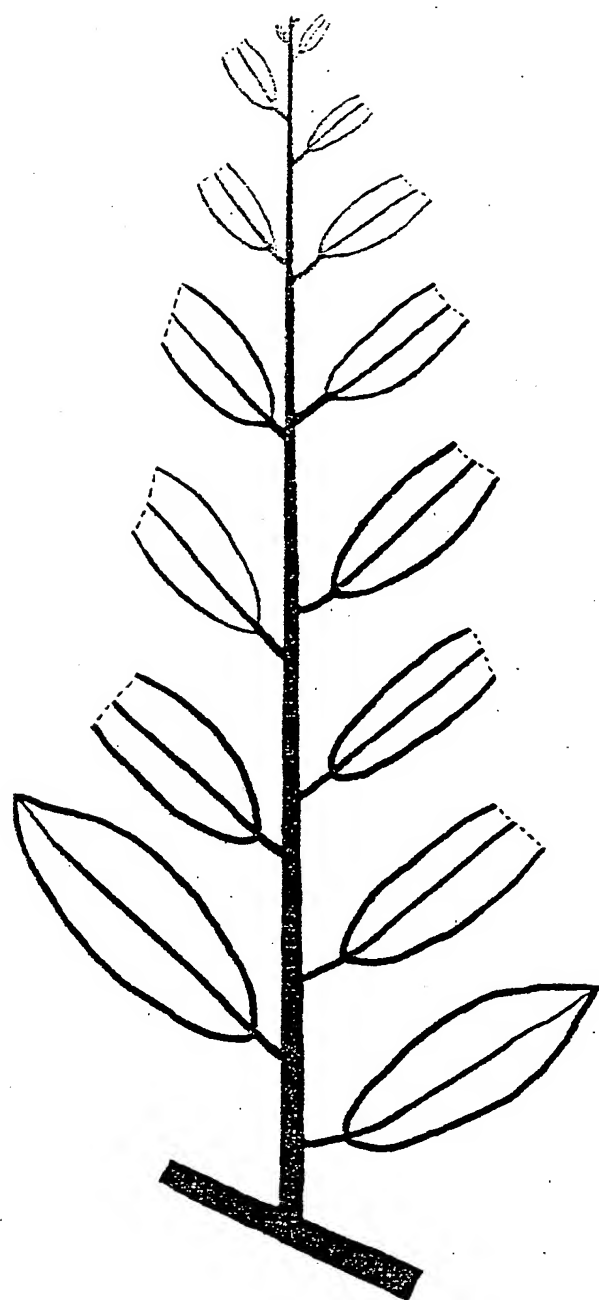
**Fig. 7**



Drawing of a *Camptotheca* seedling T-pruning treatments and control.

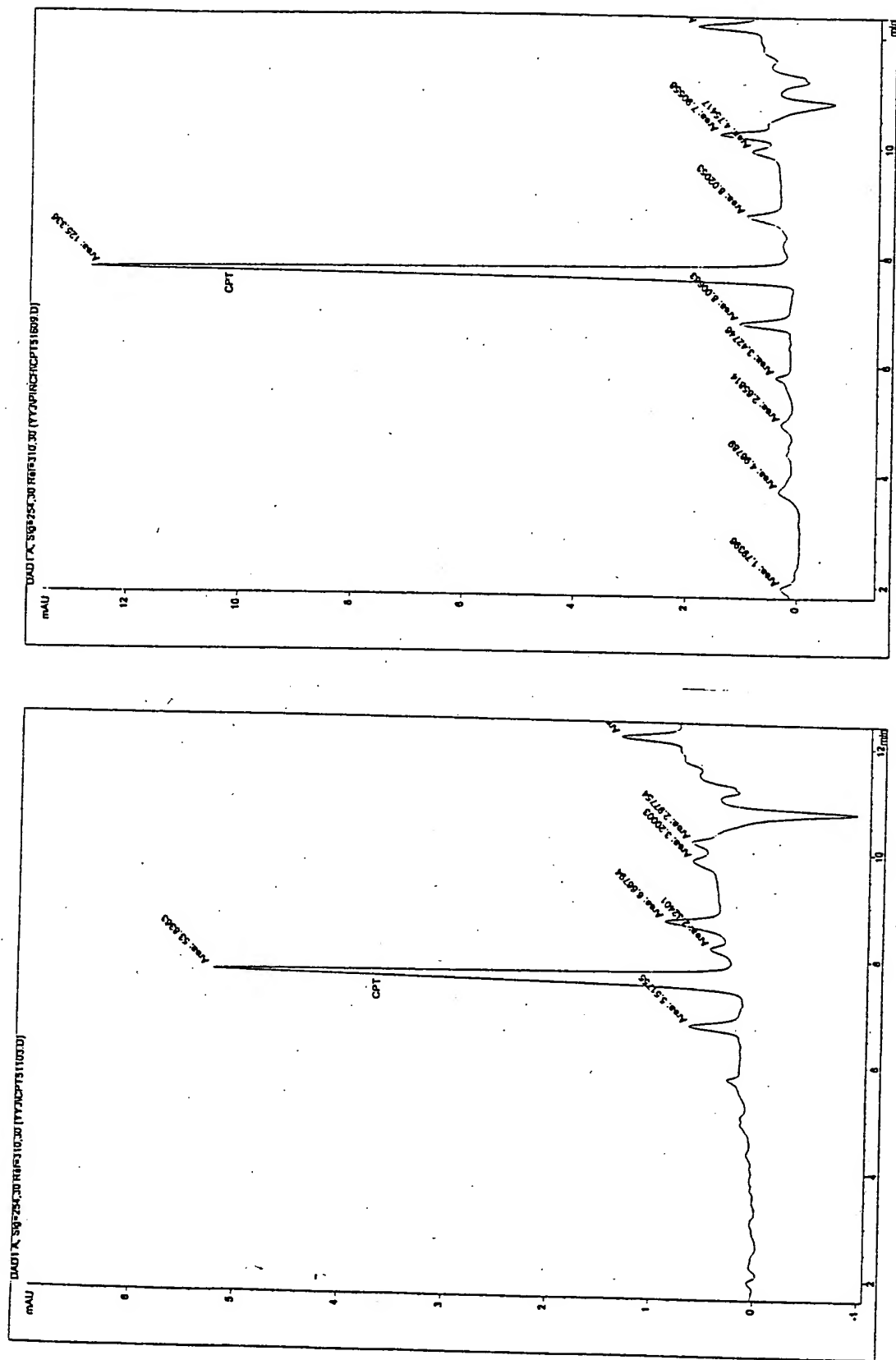
**Fig. 8**





Drawing of the leaf-tip pinching technique as applied in Camptotheca.

**Fig. 9**



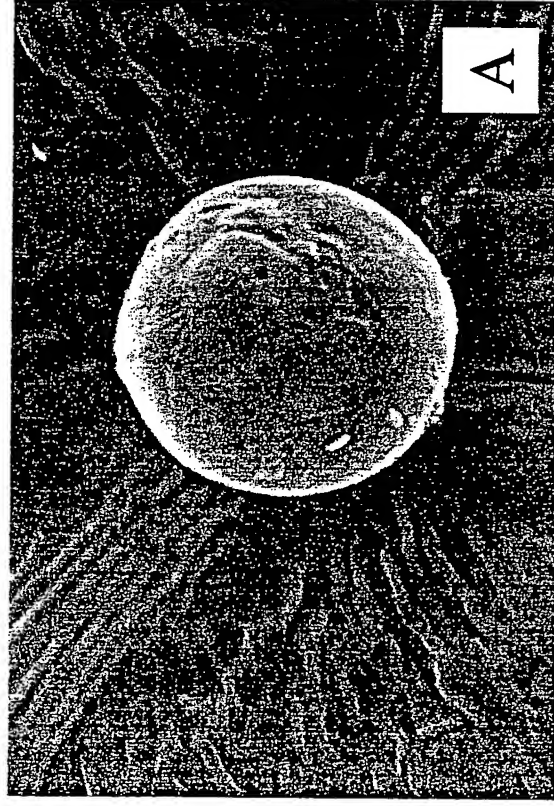
Picture of HPLC profiles showing the induction of CPT and its analogs in *Camptotheca acuminata* by pinching (a: control, b: pinching treatment).

a

b

**Fig. 10**

# Trichome Treatment



Before Treatment



After Treatment

Scanning electron micrograph of glandular trichome on upper leaf surface of Camptotheca acuminata.

**Trichome on upper leaf surface of *Camptotheca acuminata***

**Fig. 11**

	Control (cm)	Treatment I (30 cm)	Treatment II (40 cm)
Before treatment (March 25, 1997)	52.61 ± 5.44	52.15 ± 3.57	50.72 ± 6.12
After treatment (March 25, 1997)	52.61 ± 5.44	30.00 ± 0.00	40.00 ± 0.00
Net Growth (March 25-July 12, 1997)	40.10 ± 8.86 a	39.59 ± 9.73 a	32.93 ± 7.82 b
Net Growth (July 12-Sept. 13, 1997)	19.52 ± 11.39 a	31.87 ± 8.41 b	32.00 ± 9.52 b

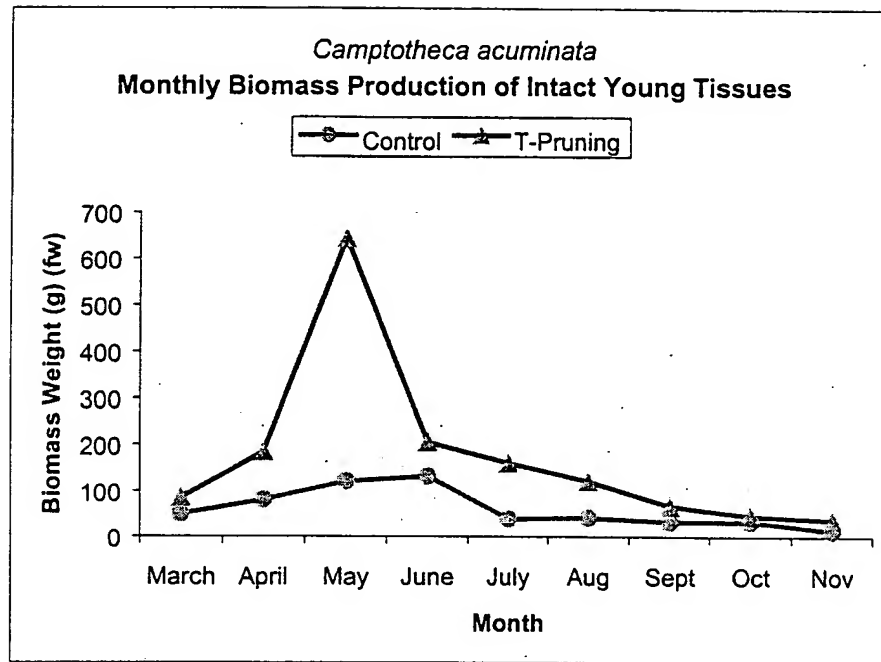
Mean height growth of plants with different T-pruning treatments (mean ± s.d.) (means with the same letter are not significantly different at  $\alpha=0.05$ ).

**Fig. 12**

	Control	Treatment I (30 cm)	Treatment II (40 cm)
Before treatment (March 25, 1997)	1.03 ± 0.17	1.04 ± 0.27	1.03 ± 0.17
After treatment (March 25, 1997)	1.03 ± 0.17	1.00 ± 0.00	1.00 ± 0.00
July 12, 1997	10.14 ± 3.06 a	10.21 ± 3.60	10.04 ± 3.16
Sept. 13, 1997	10.56 ± 3.34 a	17.00 ± 5.82 b	16.33 ± 5.21 b

**Mean branch number of plants with different T-pruning treatments (mean ± s.d.)** (means with the same letter are not significantly different at  $\alpha=0.05$ ).

**Fig. 13**



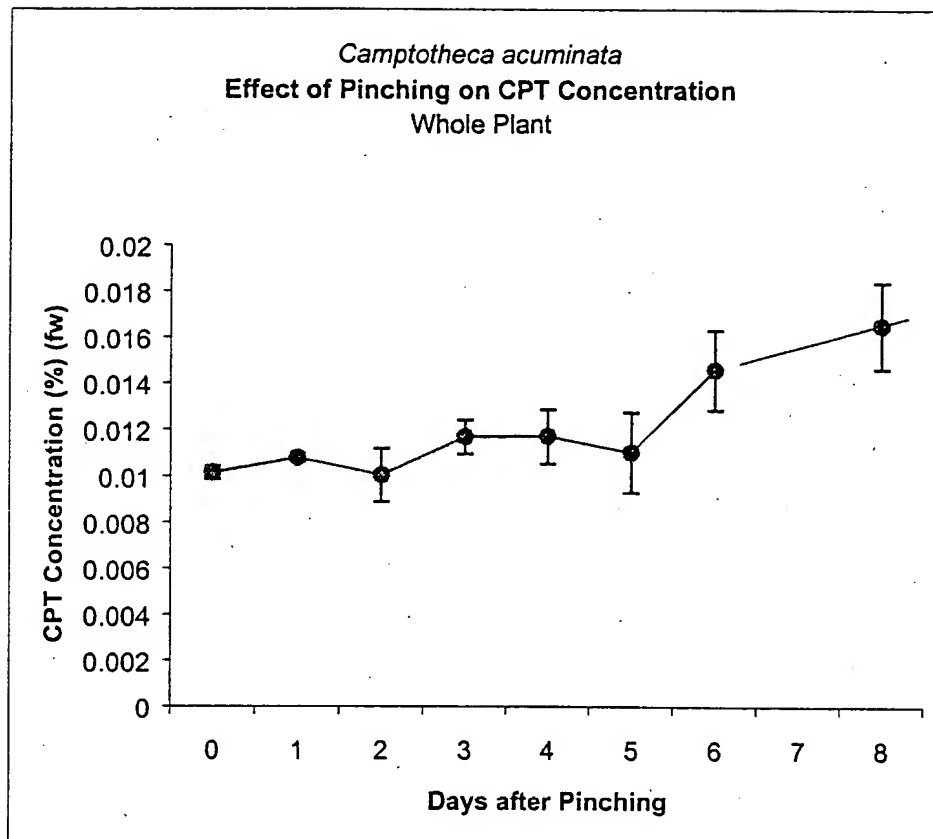
Graph of the monthly biomass production of intact young tissues with and without T-pruning

**Fig. 14**

	Control	Treatment I (30 cm)	Treatment II (40 cm)
CPT Content (%)	1.0164 ± 0.00141 a	0.0351 ± 0.0020 b	1.0437 ± 0.0037 c

Effects of T-pruning treatments on CPT contents (%) of intact young tissues of Camptotheca acuminata (mean ± s.d.) (means with the same letter are not significantly different at  $\alpha=0.05$ ) (samples were collected on June 20, 1998) (fresh weight).

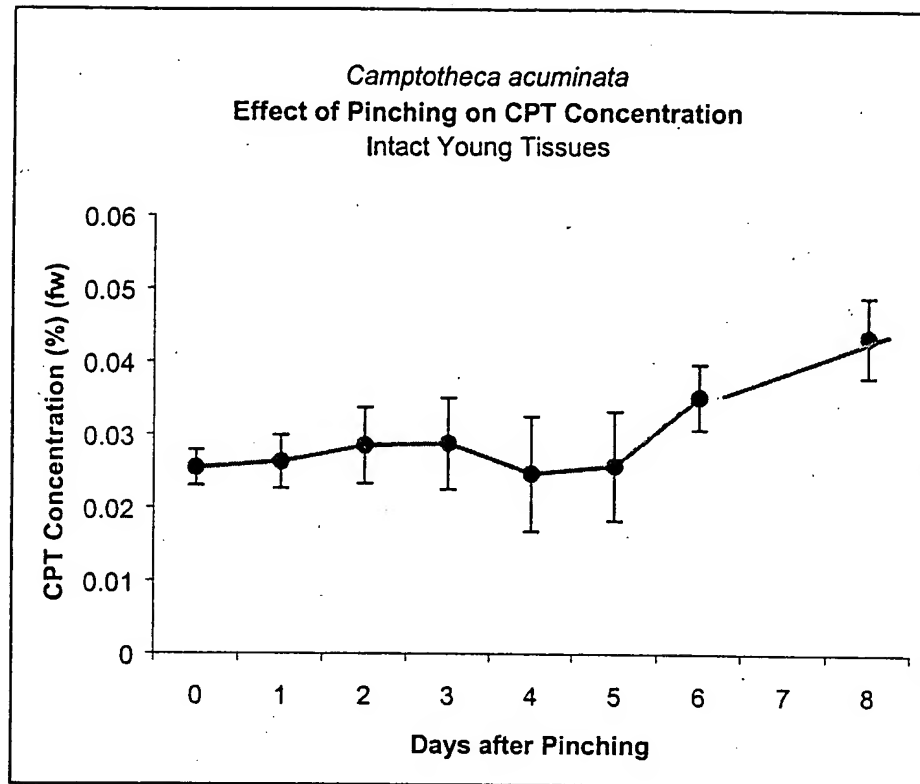
**Fig. 15**



Graph of the effect of pinching on CPT concentration in the whole plant of *Camptotheca acuminata*.

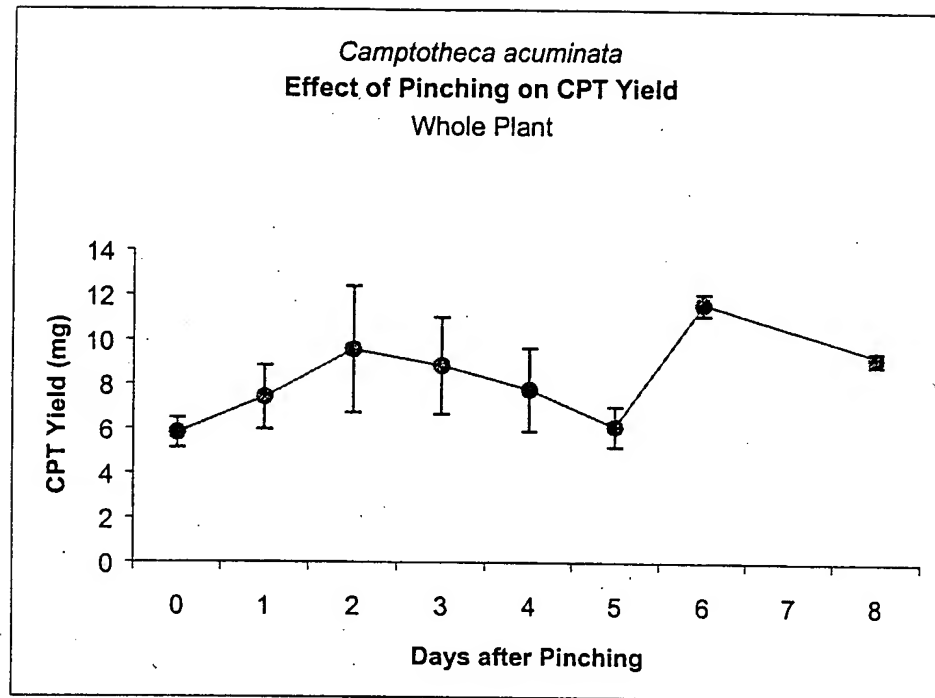
**Fig. 16a**





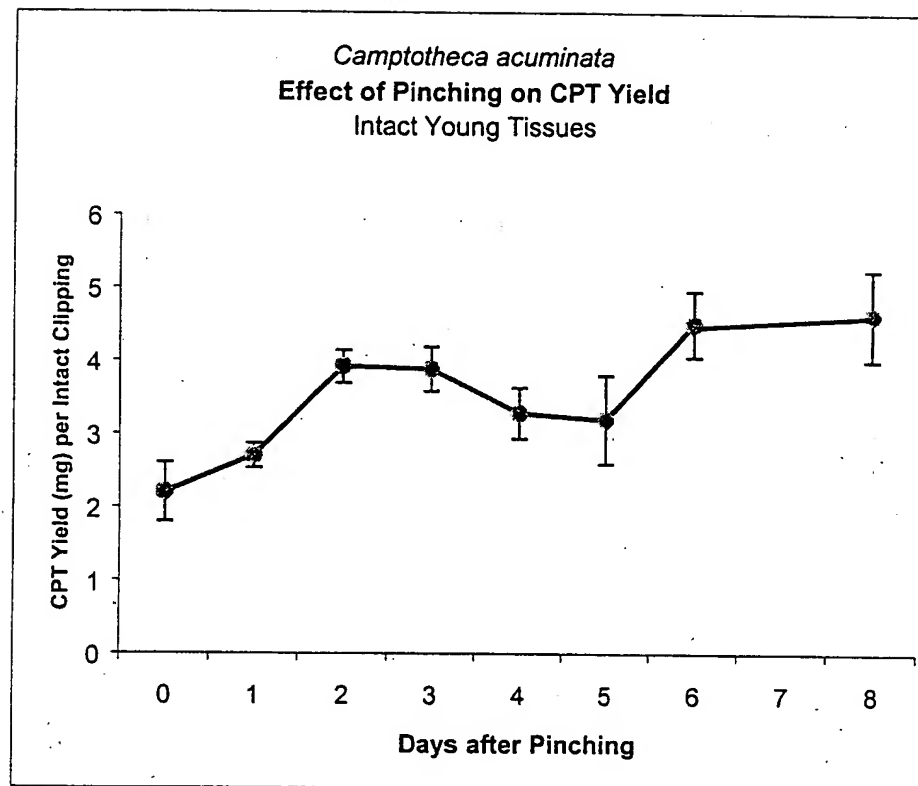
Graph of the effect of pinching on CPT concentration in intact young tissues of Camptotheca acuminata.

**Fig. 16b**



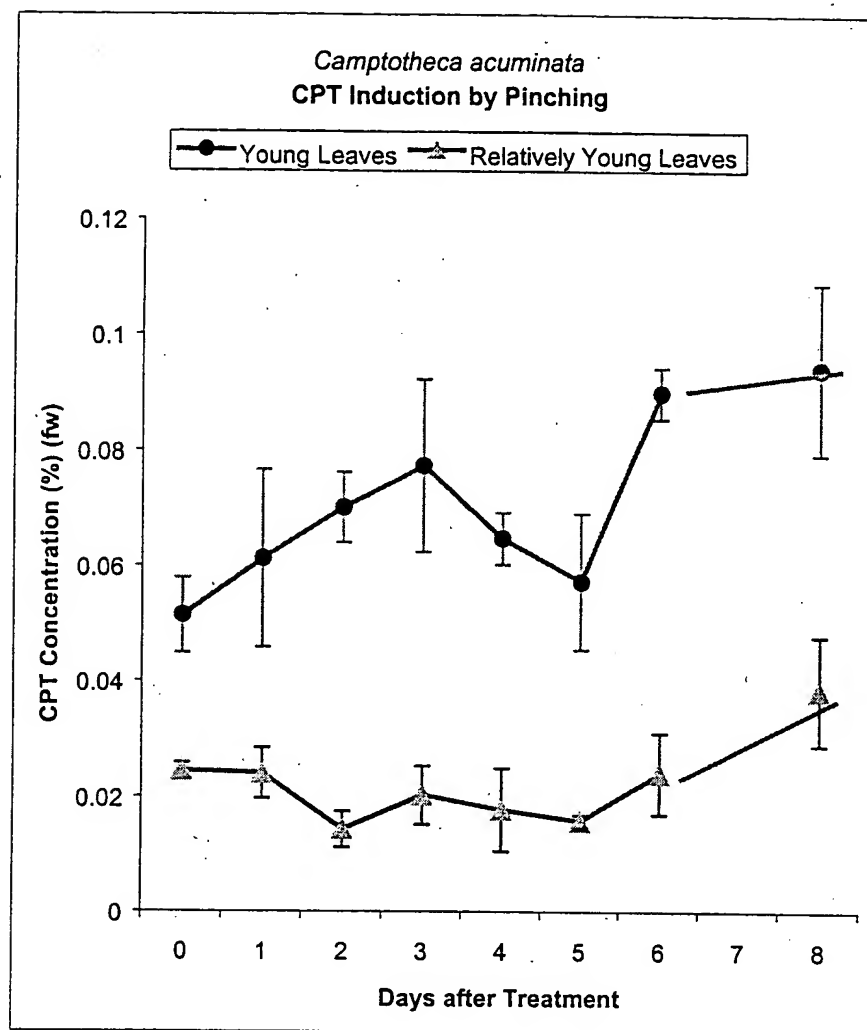
Graph of the effect of pinching on CPT yield on the whole plant in Camptotheca acuminata.

**Fig. 16c**



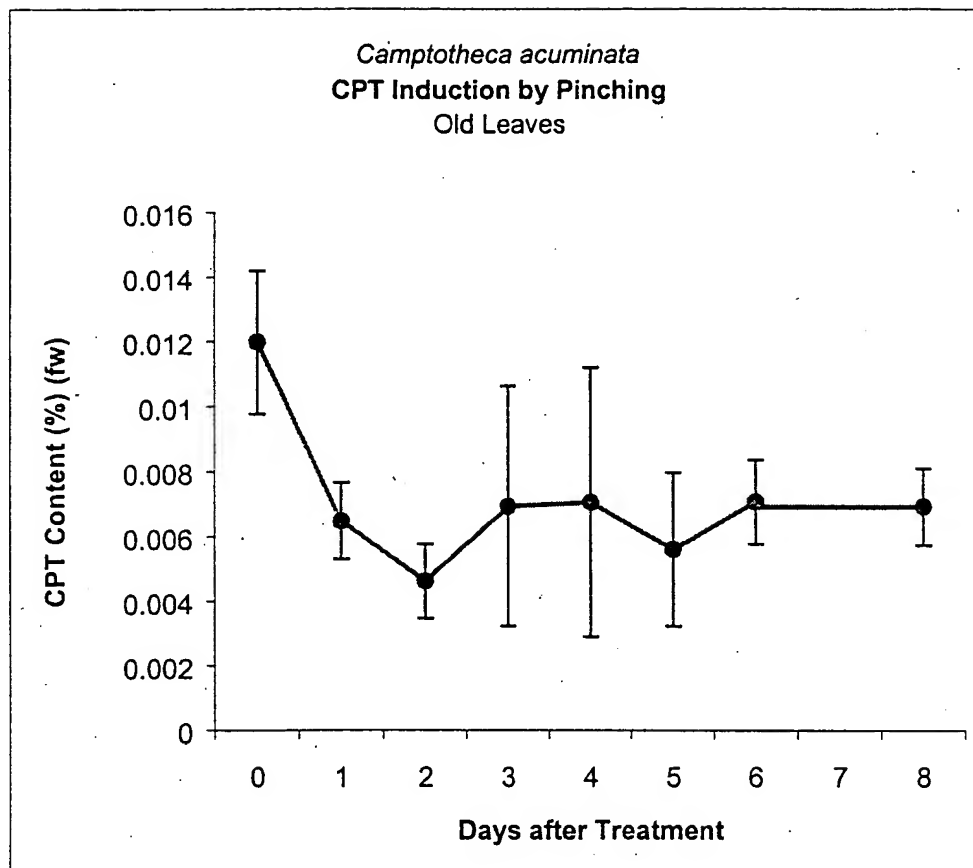
Graph of the effect of pinching on CPT yield on intact young tissues in *Camptotheca acuminata*.

**Fig. 16d**



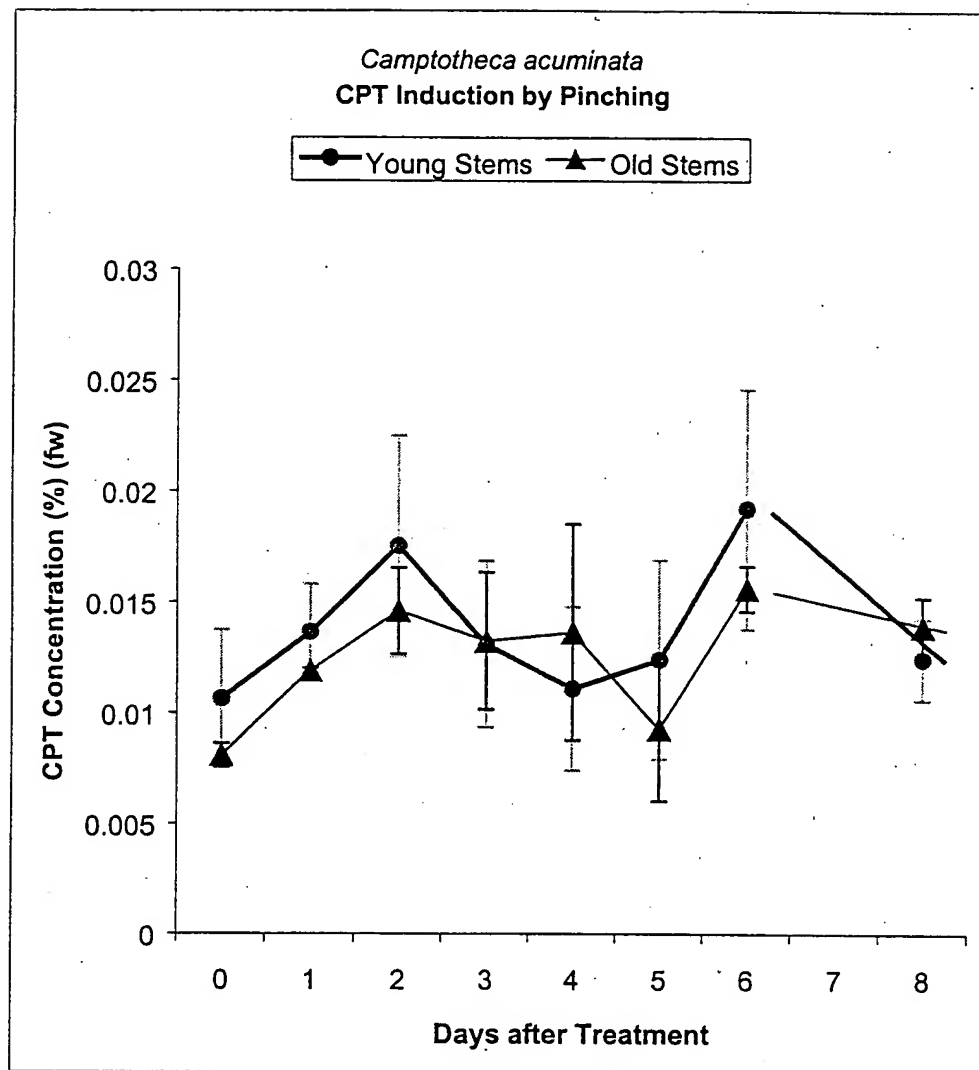
Graph of CPT induction by pinching in young leaves and relatively young leaves of *Camptotheca acuminata*.

**Fig. 17a**



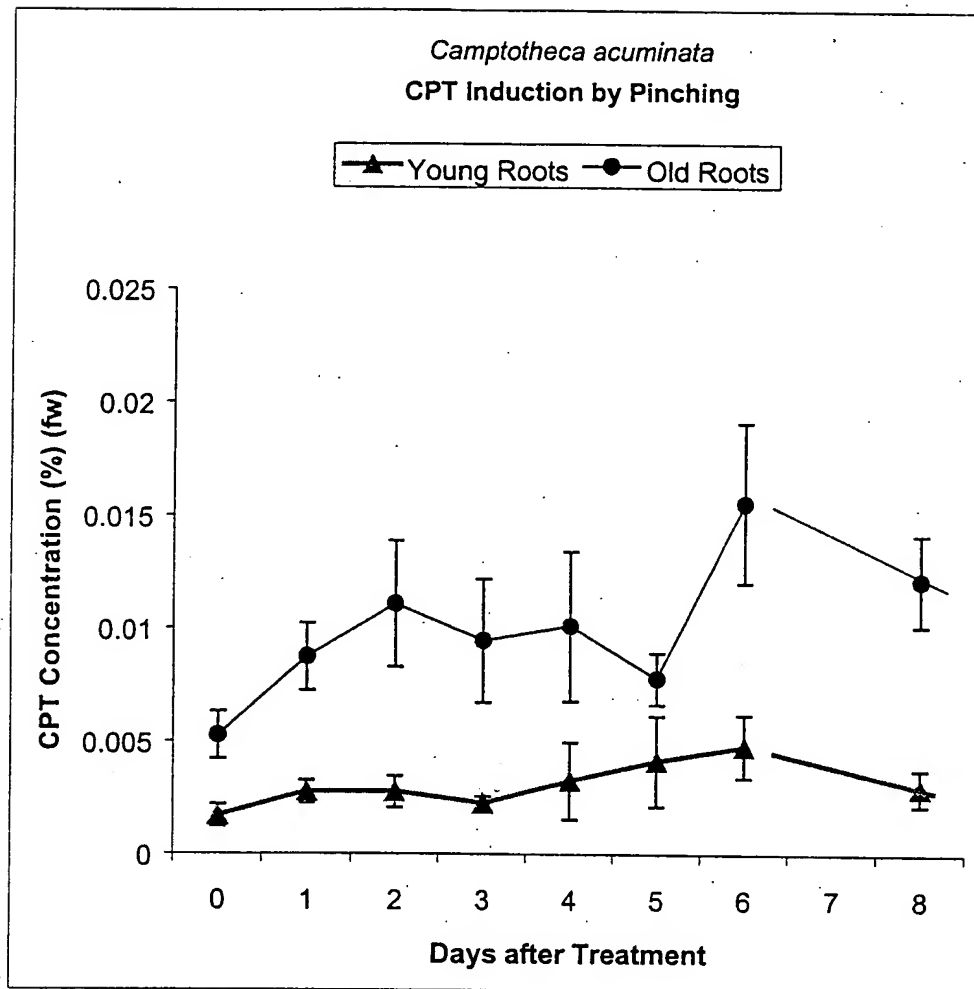
Graph of CPT induction by pinching old leaves of Camptotheca acuminata.

**Fig. 17b**



Graph of CPT induction by pinching in young stems and old stems in Camptotheca acuminata.

**Fig. 17c**



Graph of CPT induction by pinching in young roots and old roots in *Camptotheca acuminata*.

**Fig. 17d**

Treatment	CPT Concentration (%)
Pruning only	1.02506 ± 0.00389
Pruning + Pinching	0.03043 ± 0.00129

Effects of pinching treatments on CPT contents (%) of intact young tissues of Camptotheca acuminata under irrigation system (mean ± s.d.) (means with the same letter are not significantly different at  $\alpha=0.05$ ) (samples were collected on August 30, 2000) (fresh weight).

**Fig. 18**



Light Levels	Sample Size	Height	Living Branch Number
Full Sunlight	25	52.78 $\pm$ 13.08 a	1.12 $\pm$ 0.33 a
Shade	23	82.23 $\pm$ 14.51 b	2.26 $\pm$ 0.52 b

Mean growth of one-year-old seedlings grown under different light levels (mean  $\pm$  s.d.) (means with the same letter are not significantly different at  $\alpha=0.05$ ) (data were collected on July 12, 1998).

**Fig. 19**

Light Levels	Sample Size	Height	Living Branch Number	Glandular Trichome Density (no./mm <sup>2</sup> )
Full Sunlight	19	377.89 ± 59.99	17.05 ± 5.08	52.16
Shade	18	110.61 ± 21.92 b	3.67 ± 1.46	78.23 b

Mean growth and glandular trichome density of three -year-old seedlings grown under different light levels (mean ± s.d.) (means with the same letter are not significantly different at  $\alpha=0.05$ ) (data were collected on July 15, 1998.

**Fig. 20**

Treatment	Biomass (g) (fw)	CPT Concentration (%) (fw)	CPT yield (mg)
Natural Dry Condition	74.60 ± 17.74	0.05041 ± 0.00940 a	37.2112 ± 9.8481 a
Under Irrigation	213.29 ± 39.15 b	0.02754 ± 0.00648 b	59.2765 ± 22.0071 a

**Production of biomass and CPT of intact young tissues under different water conditions (mean ± s.d.)** (means with the same letter are not significantly different at  $\alpha=0.05$ ) (data were collected on August 30, 2000).

**Fig. 21**

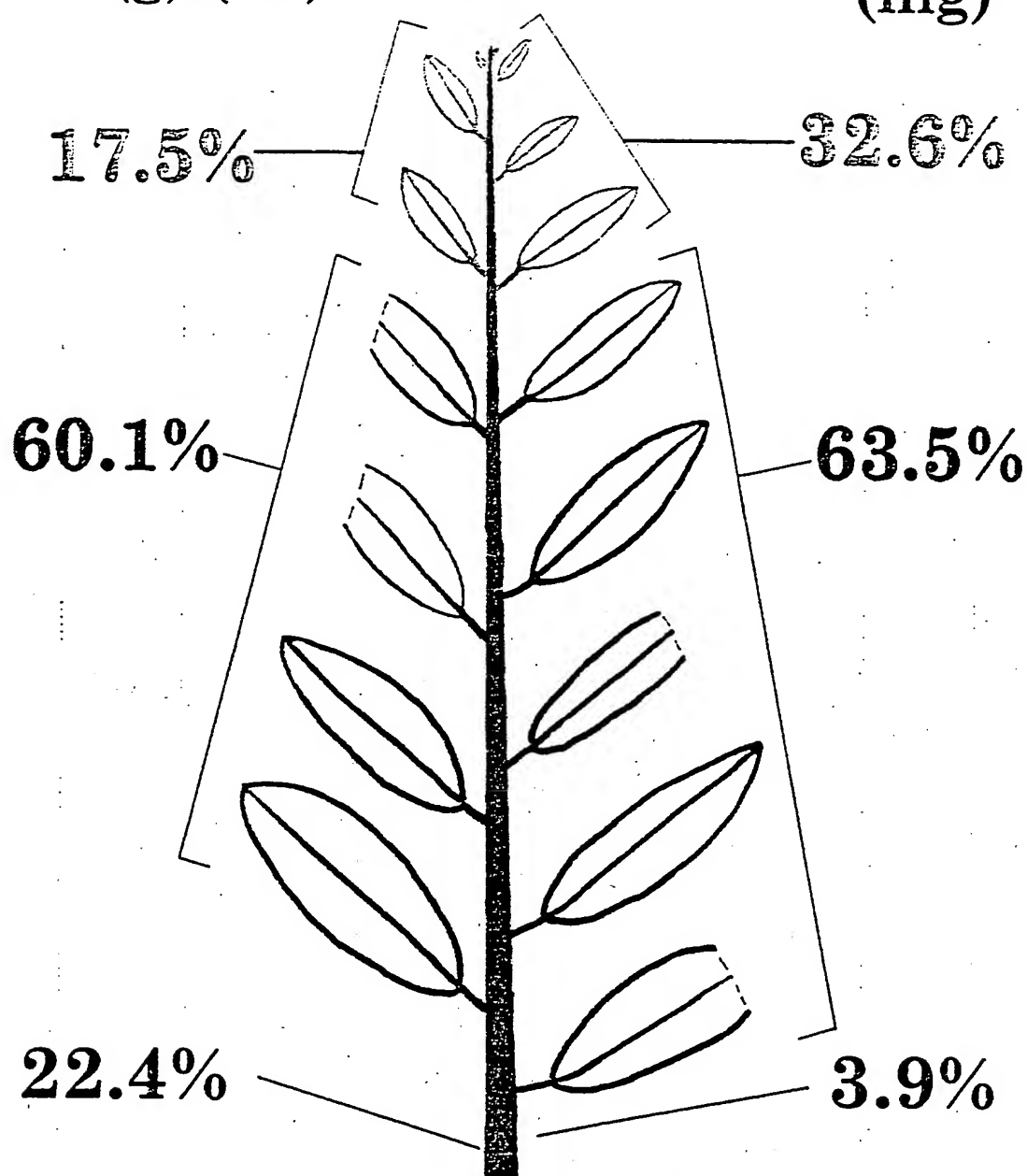
	Young Leaves	Relatively Young Leaves	Young Stems	Intact Clipping
Biomass (g) (fw)	2.2485 ± 0.1039 a	7.7315 ± 0.1506 b	2.8950 ± 1.6334	12.8950 ± 1.3789
CPT Content (%) (fw)	0.0380 ± 0.0053 a	0.0214 ± 0.0031 b	0.0080 ± 0.0030 c	0.0203 ± 0.0008
CPT Yield (mg)	0.8504 ± 0.0806 a		0.1002 ± 0.1007 c	2.6054 ± 0.1804

**Distribution pattern of biomass, CPT content, and CPT yield in an intact clipping (mean ± s.d.)** (means with the same letter are not significantly different at  $\alpha=0.05$ ) (Samples were collected on May 4, 2000).

**Fig. 22**

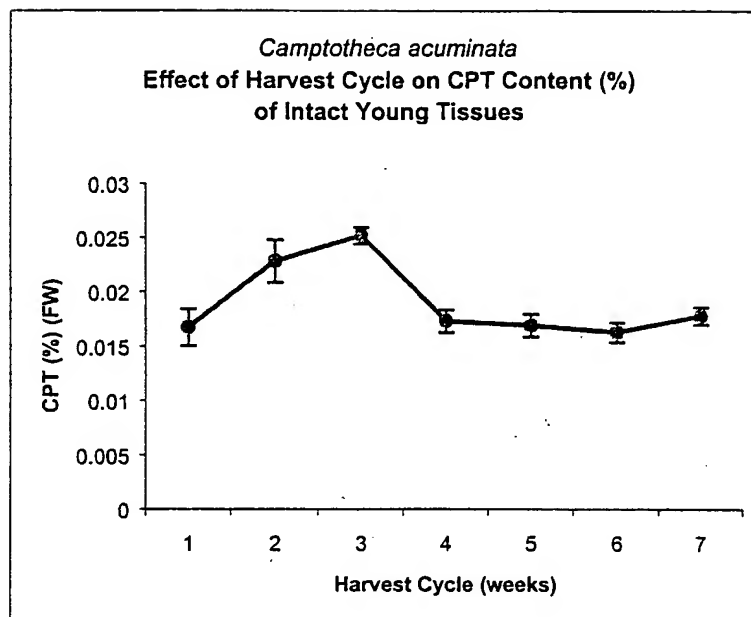
**Biomass Yield  
(g) (fw)**

**CPT Yield  
(mg)**



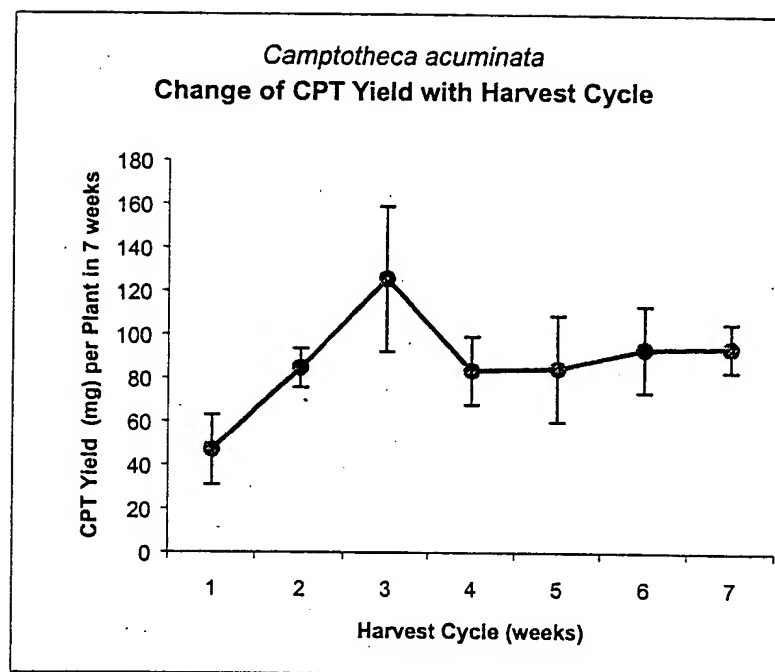
**Total CPT Yield: 2.6 mg/Intact Clipping**

**Fig. 23**



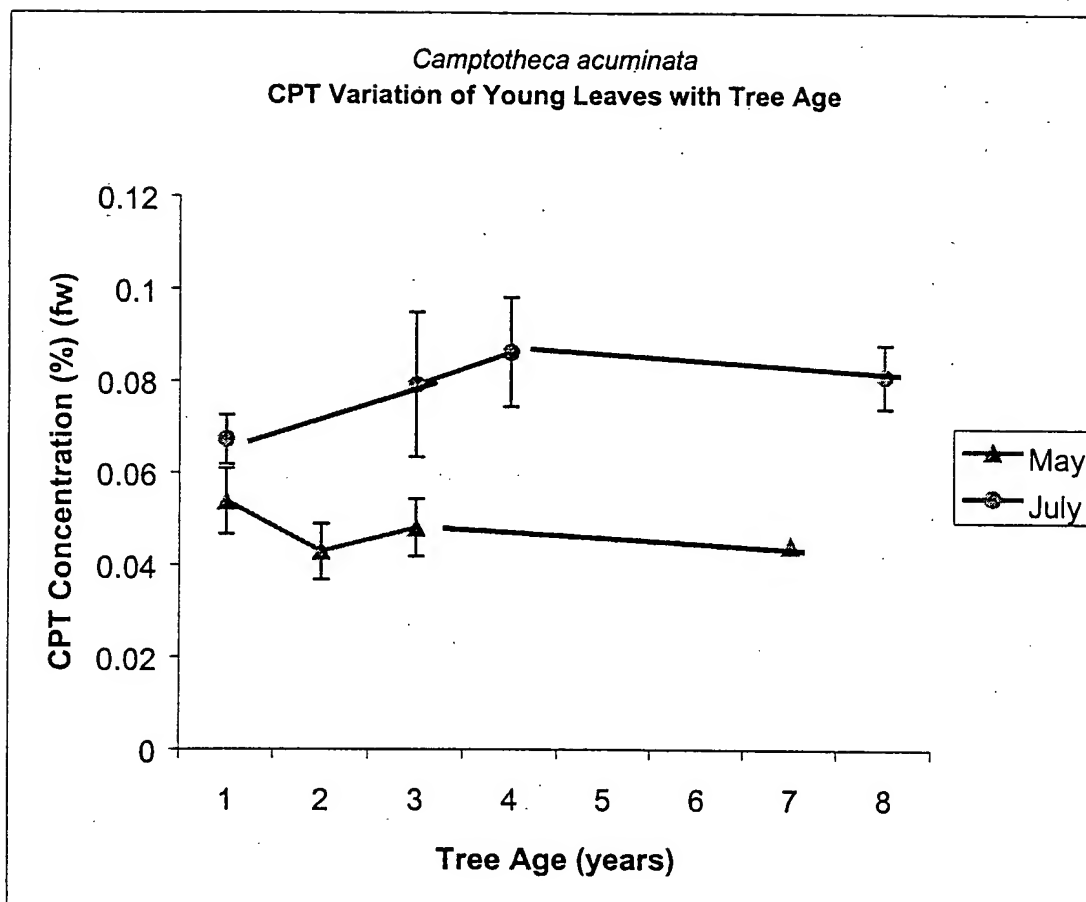
Graph of the effect of harvest cycle on CPT content of intact young tissues in Camptotheca acuminata.

**Fig. 24a**



Graph of the effect of harvest cycle on CPT yield of intact young tissues in Camptotheca acuminata.

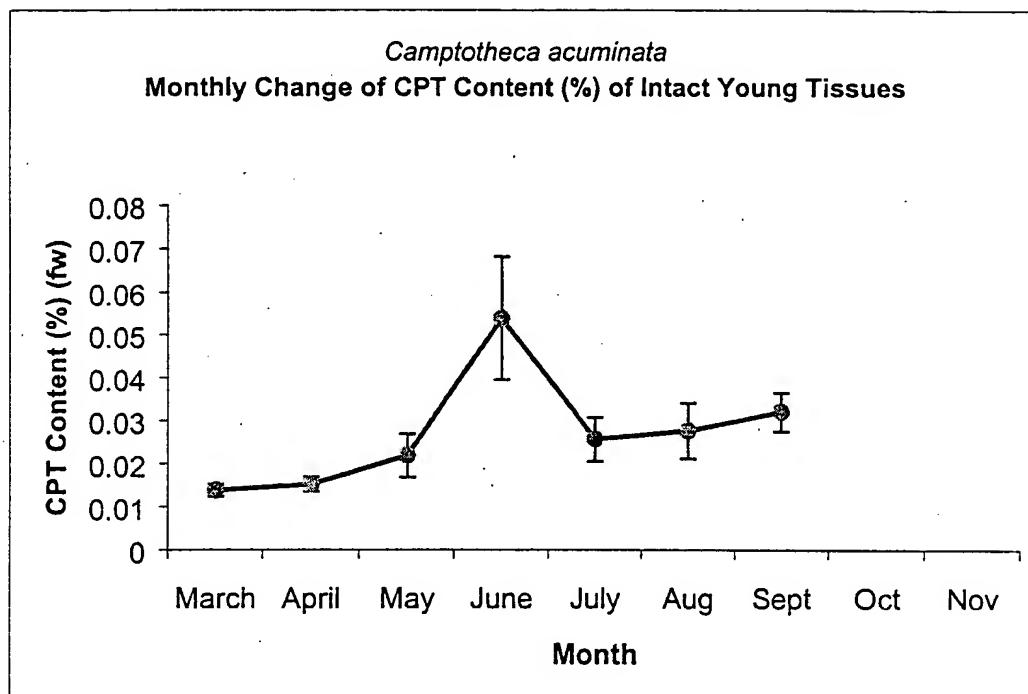
**Fig. 24b**



Graph of the variation in CPT concentration of young leaves with tree age in *Camptotheca acuminata*.

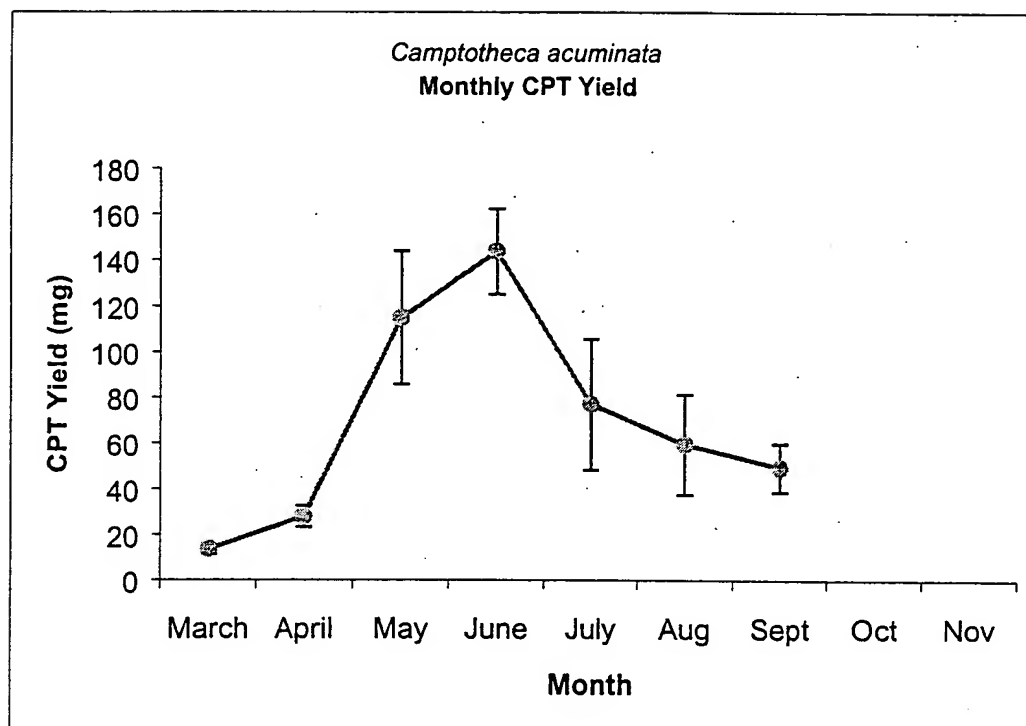
**Fig. 25**





Graph of the monthly change of CPT content of intact young tissues of Camptotheca acuminata.

**Fig. 26a**



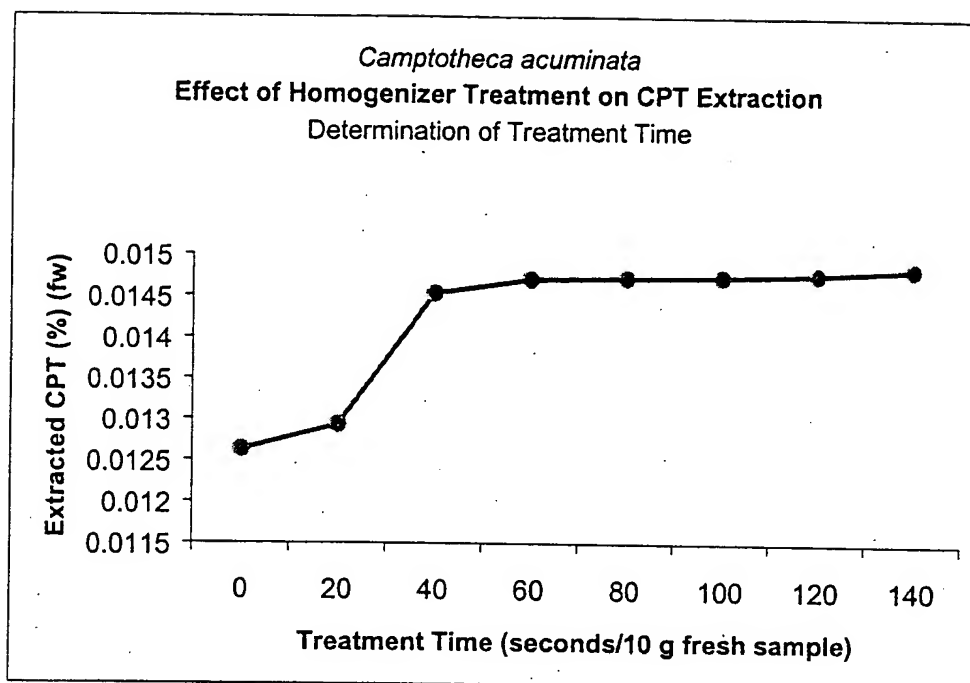
Graph of the monthly yield of CPT of intact young tissues of *Camptotheca acuminata*.

**Fig. 26b**

Preservation Method	CPT Content (% $\pm$ s.d.)
Fresh	0.03433 $\pm$ 0.0080
Freeze	0.03494 $\pm$ 0.0074
Vacuum-dry	0.03124 $\pm$ 0.00559
Air-dry	0.03015 $\pm$ 0.00797
Oven-dry (65°C)	0.02715 $\pm$ 0.0061

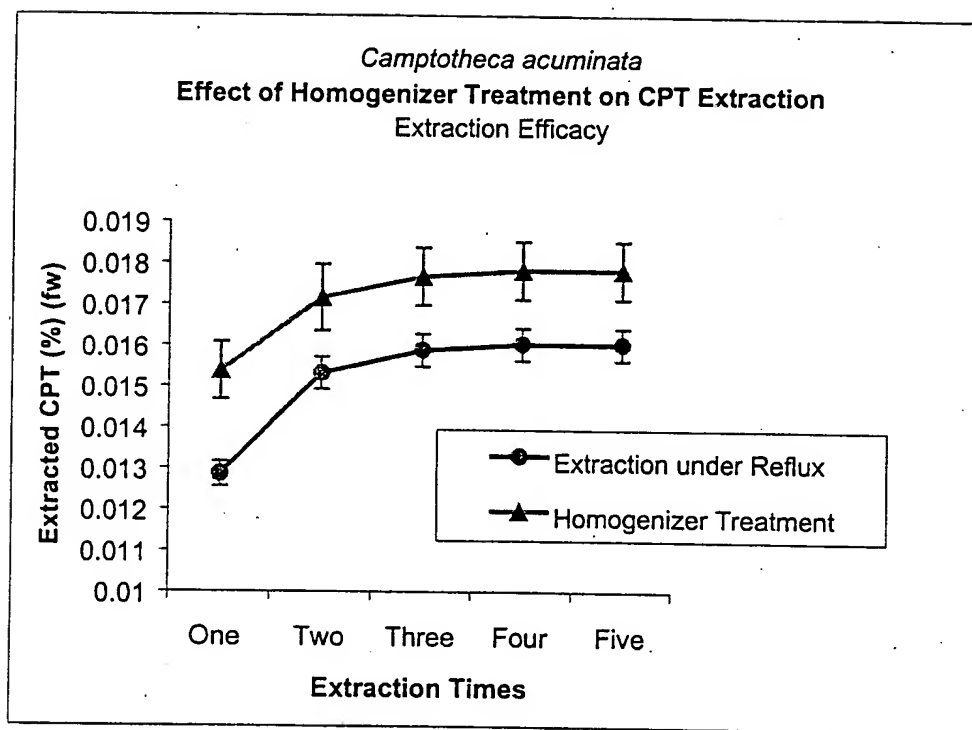
**CPT Preservation of intact young tissues preserved by different methods (samples were collected on May 31, 2000) (6 replications, fresh weight).**

**Fig. 27**



Graph of the effect of homogenizer treatment on CPT extraction in Camptotheca acuminata by duration of treatment time.

**Fig.28a**



Graph of the effect of homogenizer treatment on CPT extraction in Camptotheca acuminata by extraction efficacy.

**Fig. 28b**